



## Comparison of sensory and motor blocking action of lidocaine and lidocaine-tramadol following brachial plexus block in sheep

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### ABSTRACT

The aim of the present study was to evaluate the effect of adding tramadol to lidocaine for brachial plexus block in sheep. Six healthy, adult ewes weighing  $41.7 \pm 3.82$  kg were used. Using an electrical stimulator, brachial plexus block was performed in the sheep with one of the treatments of lidocaine (4 mg/kg) (LID), lidocaine (4 mg/kg)-tramadol (2 mg/kg) (LTL) and lidocaine (4 mg/kg)-tramadol (4 mg/kg) (LTH). All sheep received the three treatments with one-week interval. The final volume of administered solutions was adjusted to 0.3 mL/kg. Time to the onset and duration of anesthesia as well as changes in heart rate, respiratory rate and rectal temperature were determined. Time to the onset of sensory blockade and motor blockade was not significantly different among groups. The duration of sensory blockade and motor blockade was significantly longer in LTH ( $128.3 \pm 9.7$  and  $151.5 \pm 21.5$  min, respectively) compared with those of LID ( $88.6 \pm 6.5$  and  $110.5 \pm 21$  min, respectively) and LTL ( $51.6 \pm 11.8$  and  $89.6 \pm 22.7$  min, respectively). Although the onset of sensory blockade was longer than that of motor blockade in the three treatments, the difference was only significant in LTL. No significant differences were observed in heart rate, respiratory rate and rectal temperature among and within treatments. It was concluded that addition of tramadol (4 mg/kg) to lidocaine, without altering the onset, can provide more prolonged anesthesia than that of lidocaine in brachial plexus block in sheep.

### Keywords

*Brachial plexus block, Lidocaine, Nerve stimulator, Sheep, Tramadol*

### Abbreviations

BPB: Brachial Plexus Block  
HR: Heart rate  
fR: Respiratory rate  
RT: Rectal temperature  
SB: Sensory blockade  
MB: Motor blockade

## Introduction

Local and regional anesthesia are preferred over general anesthesia in ruminants. These techniques provide a cost-effective analgesia, require less monitoring and minimize considerable adverse effects suggesting a reliable alternative to general anesthesia in ruminants [1]. Brachial plexus block (BPB) is a regional anesthesia technique which has been described in cattle [1-3], goat [4] and sheep [5-9]. BPB produces analgesia in the thoracic limb to and below the elbow [1]. Ventral roots of the sixth, seventh and eighth cervical nerves (C6, C7 and C8) as well as the first and second thoracic nerves (T1 and T2) are desensitized in BPB in sheep [10].

Lidocaine is the mostly used local anesthetic in veterinary medicine. Rapid onset, intermediate duration of action and moderate toxicity has made this drug as a choice for local and regional anesthesia in animals [11]. The mean duration of action of lidocaine in BPB in sheep has been reported as 100 min [8,9]. Prolonging the duration of anesthesia and/or analgesia is favorable because it reduces the need for re-administration of lidocaine and provides long-lasting postoperative analgesia. Various pharmacologic agents in combination of lidocaine have been investigated to provide more prolonged analgesia in BPB. Epinephrine, xylazine, morphine and tramadol have been investigated as adjuvants to lidocaine for BPB in sheep [8,9]. Among these additives, only xylazine increased the duration of both sensory and motor blockade of BPB; however, it was associated with sedation, bradycardia and increased urination [8].

Tramadol, an atypical opioid, is widely used to treat or relieve acute or chronic pain. In addition to systemic analgesic impacts, tramadol has been postulated to have some local anesthetic effects [12,13]. Availability, different mechanisms of action and lower incidence of serious adverse effects of tramadol have made this drug as an agent of interest for local and regional anesthesia. Using of tramadol alone or in combination with lidocaine has increased the duration of analgesia after epidural application in ruminants [14-17]. Tramadol (in a range of 100-200 mg) when added to different local anesthetics has increased the quality and duration of BPB in human studies [18-24]. In a recent study in sheep, addition of 1 mg/kg tramadol to lidocaine for BPB did not increase the duration of neither sensory nor motor blockade in comparison to those of lidocaine alone [9]. Since tramadol has been speculated that induces analgesia in a dose-dependent manner [14], employing of larger doses of tramadol might produce more prolonged analgesia following BPB in sheep. Thus, the objective of the present study was to evaluate the effect of tramadol (2 and 4 mg/kg) in combination with lidocaine on BPB in sheep. We

hypothesized that using of these doses could increase the duration of anesthesia without serious adverse effects.

## Result

There was no significant difference with respect to body weights among groups: LID:  $41.4 \pm 4.5$ , LTL:  $40.4 \pm 4.5$  and LTH:  $43.5 \pm 2.5$ . Overall, All the sheep tolerated the procedure well; however, movement and unrest at the time of needle advancement were occasionally seen. The proper site for administration of treatments was easily identified and all the aspirations were negative. BPB was failed in one sheep in LTL and one sheep in LTH. Successful BPB was achieved by the repetition of the procedure in the aforementioned sheep one week later. Injection of drugs took a time of 3-6 min in all groups. The pH of the administered solutions was measured as  $6.34 \pm 0.02$  for LID,  $6.33 \pm 0.04$  for LTL and  $6.33 \pm 0.02$  for LTH.

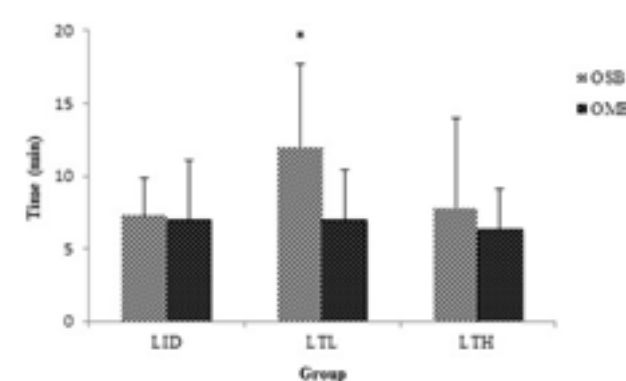
Time to the onset of sensory and motor blockade was not significantly different among groups ( $p > 0.05$ ). Although the onset of sensory blockade in comparison to the onset of motor blockade was longer in the three treatments, the difference was only significant in LTL ( $p < 0.05$ ; Figure 1). The duration of loss of sensation was significantly longer in LID than that of LTL ( $p < 0.05$ ). The duration of sensory and motor blockade was significantly longer in LTH compared to those of LID and LTL ( $88.6 \pm 6.5$  and  $110.5 \pm 21$  min for LID,  $51.6 \pm 11.8$  and  $89.6 \pm 22.7$  min for LTL and  $128.3 \pm 9.7$  and  $151.5 \pm 21.5$  min for LTH) ( $p < 0.05$ ; Figure 2).

Statistical analysis of HR, fR and RT did not show significant differences among and within treatments ( $p > 0.05$ ; Table 1). No sedation and adverse reactions and no signs of local anesthetic's toxicity were seen following BPB in any of the sheep. Some degree of hemorrhage was observed in the left lateral wall of the thorax in one out of two sheep which were slaughtered two weeks after the end of last treatment.

## Discussion

Adjuvants are used to increase the quality and duration of anesthesia induced by local anesthetics. A desirable adjuvant should provide better anesthetic characteristics; meanwhile, it should have less adverse effects. Based on the results of the present investigation, addition of 4 mg/kg tramadol to lidocaine for BPB, without altering the onset of action, prolongs the duration of sensory and motor blockade in sheep. Moreover, tramadol in combination with lidocaine did not result in noticeable side effects in sheep undergoing BPB.

Tramadol has been suggested that exerts its pe-



**Figure 1.**

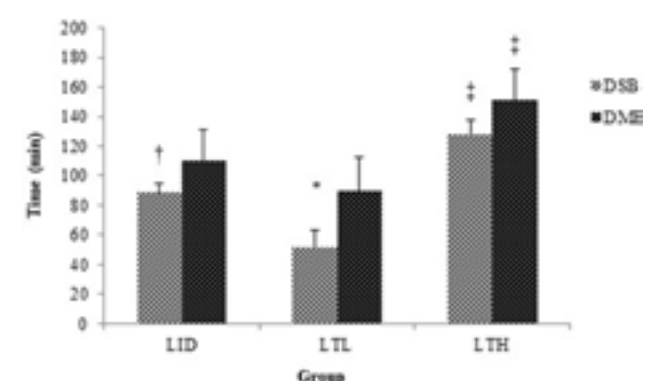
Mean and SD time to onset of sensory and motor blockade (OSB and OMB, respectively) using lidocaine (4 mg/kg; LID), lidocaine (4 mg/kg) plus low dose of tramadol (2 mg/kg; LTL) and lidocaine (4 mg/kg) plus high dose of tramadol (4 mg/kg; LTH) for brachial plexus block in sheep (n = 6)

\*Significantly different from OMB in LTL.

ripheral anesthetic properties through various mechanisms. Blockade of opioids and  $\alpha$ -2 agonist receptors [25], blockade of sodium and potassium channels in the cell membranes [26-29], and blockade of peripheral nerve conduction [13,29] have been proposed for possible local anesthetic's characteristics of tramadol. Longer duration of analgesia as a result of systemic effects of tramadol following local application of tramadol and levobupivacaine has also been reported in human patients undergoing shoulder arthroplasty. However, the same effects have not been observed after systemic administration of tramadol [30].

Conflicting results have been obtained with respect to the onset of action after addition of tramadol to local anesthetics in human's axillary blocks. While someone have reported faster onset of anesthesia [18,19,22,23], others have documented a delay in the initiation of action [20,24]. Several authors also failed to detect any changes in the onset of anesthesia [21,23,31]. A study in sheep demonstrated that 1 mg/kg tramadol did not alter the onset of sensory and motor blockade when used in combination with lidocaine for BPB [9].

Duration of anesthesia was significantly longer in sheep received larger dose of tramadol (i.e. 4 mg/kg). In human studies, addition of tramadol in a range of 100-200 mg to different local anesthetics has increased the duration of action of BPB [18-24]. Ghadirian et al. [9] did not observe increase in the duration of sensory and motor blockade after addition of 1 mg/kg tramadol to lidocaine for BPB in sheep. Considering the sheep's weights in the latter study (i.e.  $27.0 \pm 2.2$  mg), the authors have used tramadol with the total dose of  $27.0 \pm 2.2$  mg which is much less than that of human's investigations. In the current study, the total doses of tramadol were  $80.8 \pm 9.0$  and  $173.2 \pm 10.0$  mg in LTL



**Figure 2.**

Mean and SD duration of sensory and motor blockade (DSB and DMB, respectively) using (4 mg/kg; LID), lidocaine (4 mg/kg) plus low dose of tramadol (2 mg/kg; LTL) and lidocaine (4 mg/kg) plus high dose of tramadol (4 mg/kg; LTH) for brachial plexus block in sheep (n = 6)

† Significantly different from DSB in LTL.

‡ Significantly different from DSB and DMB in LID and LTL.

\*Significantly different from DMB in LTL.

and LTH, respectively. While several factors such as type, concentration, volume and method of application can affect the duration of anesthesia in local and regional nerve blocks [9], it seems the total dose of tramadol plays an important role in the duration of action in BPB. Therefore, although tramadol with the dose rate of 4 mg/kg increased the duration of anesthesia but the weights of the sheep is also a substantial factor to achieve a successful more prolonged block. Further studies are needed to determine the exact effective total dose of tramadol for increasing the duration of anesthesia of BPB in sheep.

In the current study, the combination of 2 mg/kg tramadol and lidocaine not only did not increase the duration of anesthesia, but also decreased both the sensory and motor blockade of BPB. However, just the duration of sensory blockade was significantly lower than that of lidocaine alone. Although it was not significant, the duration of sensory and motor blockade in the study of Ghadirian et al. [9] were also lower in lidocaine-tramadol group than those of lidocaine alone following BPB in sheep. Further studies are necessary to confirm these findings and to clarify the exact reason(s) and/or mechanism(s).

The onset of sensory blockade was longer than that of motor blockade in the three groups; however, the difference was only significant in LTL. The same results have been reported following BPB in sheep [8,9], dogs [32,33], and goats [4]. The more rapid onset of sensory blockade has been explained by somatotopical arrangement of nerve fibers in brachial plexus where motor and sensory fibers located in the periphery (mantle bundles) and center (core) of the nerve trunk, respectively [34].

HR, fR and RT did not show significant changes within the groups when compared with the base



**Table 1**  
Mean  $\pm$  SD heart rate (HR), respiratory rate (fR) and rectal temperature (RT) in sheep (n = 6) received lidocaine (4 mg/kg; LID), lidocaine plus low dose of tramadol (2 mg/kg; LTL) and lidocaine (4 mg/kg) plus high dose of tramadol (4 mg/kg; LTH) for brachial plexus block

	Base	5	10	15	20	30	45	60	75	90	105	20	50	80
	(min)	(min)	(min)	(min)	(min)	(min)	(min)	(min)	(min)	(min)	(min)	(min)	(min)	(min)
HR	LID	73 $\pm$ 16	97 $\pm$ 11	102 $\pm$ 23	96 $\pm$ 19	86 $\pm$ 22	80 $\pm$ 24	86 $\pm$ 16	75 $\pm$ 13	80 $\pm$ 12	77 $\pm$ 15	74 $\pm$ 21	75 $\pm$ 17	70 $\pm$ 14
	LTL	80 $\pm$ 15	95 $\pm$ 16	87 $\pm$ 18	91 $\pm$ 18	84 $\pm$ 15	80 $\pm$ 17	75 $\pm$ 19	73 $\pm$ 18	77 $\pm$ 15	77 $\pm$ 15	76 $\pm$ 13	71 $\pm$ 17	70 $\pm$ 15
	LTH	91 $\pm$ 16	92 $\pm$ 10	89 $\pm$ 14	91 $\pm$ 16	90 $\pm$ 8	81 $\pm$ 18	96 $\pm$ 28	89 $\pm$ 25	85 $\pm$ 22	84 $\pm$ 22	81 $\pm$ 26	80 $\pm$ 27	77 $\pm$ 23
fR	LID	40 $\pm$ 10	42 $\pm$ 7	47 $\pm$ 3	43 $\pm$ 9	43 $\pm$ 11	44 $\pm$ 13	50 $\pm$ 25	51 $\pm$ 16	42 $\pm$ 9	45 $\pm$ 11	47 $\pm$ 6	45 $\pm$ 12	36 $\pm$ 9
	LTL	46 $\pm$ 14	54 $\pm$ 18	50 $\pm$ 23	42 $\pm$ 14	39 $\pm$ 18	38 $\pm$ 15	49 $\pm$ 22	51 $\pm$ 21	47 $\pm$ 25	49 $\pm$ 23	49 $\pm$ 20	49 $\pm$ 19	47 $\pm$ 17
	LTH	40 $\pm$ 6	58 $\pm$ 19	50 $\pm$ 18	52 $\pm$ 16	50 $\pm$ 18	55 $\pm$ 15	56 $\pm$ 22	54 $\pm$ 24	56 $\pm$ 21	60 $\pm$ 26	57 $\pm$ 20	50 $\pm$ 11	50 $\pm$ 12
RT	LID	39.2 $\pm$ 0.5	39.5 $\pm$ 0.4	39.4 $\pm$ 0.4	39.3 $\pm$ 0.3	39.3 $\pm$ 0.3	39.3 $\pm$ 0.3	39.3 $\pm$ 0.3	39.2 $\pm$ 0.4	39.2 $\pm$ 0.4	39.2 $\pm$ 0.4	39.2 $\pm$ 0.4	39.3 $\pm$ 0.5	39.1 $\pm$ 0.5
	LTL	39.1 $\pm$ 0.4	39.4 $\pm$ 0.3	39.4 $\pm$ 0.4	39.3 $\pm$ 0.5	39.3 $\pm$ 0.4	39.4 $\pm$ 0.4	39.3 $\pm$ 0.3	39.3 $\pm$ 0.3	39.3 $\pm$ 0.3	39.3 $\pm$ 0.3	39.3 $\pm$ 0.2	39.2 $\pm$ 0.1	39.0 $\pm$ 0.2
	LTH	39.1 $\pm$ 0.2	39.2 $\pm$ 0.3	39.4 $\pm$ 0.3	39.2 $\pm$ 0.4	39.3 $\pm$ 0.3	39.2 $\pm$ 0.4	39.3 $\pm$ 0.4	39.4 $\pm$ 0.4	39.2 $\pm$ 0.4	39.1 $\pm$ 0.4	39.0 $\pm$ 0.3	39.0 $\pm$ 0.3	38.9 $\pm$ 0.2

values. No significant differences in HR and fR have also been reported following application of 1 mg/kg tramadol and lidocaine for BPB in sheep [9]. Addition of tramadol (100 mg) to mepivacaine for BPB in human patients did not alter HR, blood pressure and fR [21]. One of the main advantages of tramadol is its minimal effects on cardiovascular variables [35], and according to the results of the present study, tramadol is a safe adjuvant for addition to local anesthetics even when the dose rate of as high as 4 mg/kg were used for BPB in sheep.

Various methods including blind, nerve stimulator- and ultrasound-guided techniques have been described for performing BPB [36]. Since the success rate of a regional anesthesia depends on the ability to accurately locate the target nerves and to place the solution as close as possible to nerves, two latter methods offer increasing the rate of successful anesthesia. Performing BPB using nerve stimulator has raised the success rate from 20% to 75% and 95% in dogs and goats, respectively [4,33]. No differences in success rate and complications following nerve stimulator- and ultrasound-guided BPB were observed in humans [37]. In the current study, an overall success rate of 88% was recorded which is comparable with the results of the other studies reported the success rate of 86% and 89% after BPB in sheep using a nerve stimulator [8,9]. It is trustworthy to note that, in the present study, BPB was failed in two sheep which were in the higher range of weight (i.e. 44 and 45.5 kg).

The final volume of local anesthetic solution can play an important role on overall outcome of BPB (38, 39). A total volume of 10-40 mL has been proposed for BPB in cows and sheep [2,3,5]. Moens (1995) [4] used 0.3-0.4 mL/kg for performing BPB in goats. Applying of 0.25 mL/kg of anesthetic solution for BPB in sheep has been suggested as may not be enough to achieve complete anesthesia in all sheep [8,9]. In the current study, mainly because of using the large volume of tramadol, the final volume of administered solutions was adjusted to 0.3 mL/kg. However the overall rate of successful block did not increase compared with those of the aforementioned investigations (88% vs 86% and 89%).

Intravascular injection, hemorrhage, pneumothorax, lung injury, nerve injury and infection are the possible complications associated with BPB [38,40]. Administration of high doses of local anesthetics may be resulted in systemic toxicity [11]. Pruritus after application of tramadol (100-200 mg) and mepivacaine combination has been reported in humans undergoing BPB [23]. In the current study, no adverse effect related to tramadol and/or lidocaine was observed. However one out of two slaughtered sheep showed some degree of hemorrhage in the left lateral wall of

the thorax most probably due to displacing the needle to find the appropriate twitches in the limb.

Conclusion

Nerve stimulator-guided block is an acceptable method for performing BPB in sheep. Addition of tramadol (4 mg/kg) to lidocaine (4 mg/kg) in BPB, without changing the onset, increased the duration of sensory and motor blockade in comparison to lidocaine alone (4 mg/kg) in sheep. The occurrence of complications associated with BPB in sheep is rare; however, negative aspiration for blood and air is strongly recommended.

Material and methods

Six healthy, adult ewes with weight of 41.7  $\pm$  3.82 (mean  $\pm$  SD) kg and age of 1-2 (range) years old were used. The sheep were transferred to Veterinary Hospital, at least 2 weeks prior to the beginning of the investigation, to be acclimatized to the circumstances. The animals were considered to be healthy based on a thorough physical examination and normal complete blood count and total protein. The sheep were fed alfalfa, straw and grain, and water ad libitum and were not fasted before the experiments. All the procedures were performed in the evening (2:00-5:00 PM). The institutional Animal Care and Research Committee approved all the protocols of the present study [95/3/24/4550].

Sheep were assigned to receive one of the three treatments on a blinded random fashion: lidocaine (4 mg/kg; Caspian Tamin, Pharmaceutical Co, Iran) (LID), lidocaine plus low dose of tramadol (2 mg/kg; Darou Pakhsh, Iran) (LTL) and lidocaine plus high dose of tramadol (4 mg/kg) (LTH). Using normal saline, the total volume of administered drugs was fixed at 0.3 mL/kg. A digital pH meter (Suntex, Taiwan) was employed to determine the pH of solutions. Each sheep was used on three occasions with at least one-week interval. The study was designed in a way that in each group three right and three left thoracic limbs were administered.

After recording heart rate (HR), respiratory rate (fR) and rectal temperature (RT) as well as confirming of the normal sensory and motor functions of the thoracic limb, an area of about 15 cm of the skin overlying the scapulohumeral joint was aseptically prepared. Then, sheep were positioned in lateral recumbency with the target limb uppermost. Two mL lidocaine 1% was injected subcutaneously at the site of needle puncture. BPB was performed using an insulated needle (SonoPlex stim cannula, 22 G  $\times$  80 mm, Pajunk, Germany) connected to an electrical stimulator (Neurodyn710, Novin Medical Engineering CO, Iran). The method employed for BPB was based on previous studies in sheep [8, 9]. In summary, the negative electrode was connected to the insulated needle and the positive electrode was attached to the skin with about 10-cm distance from the shoulder joint. The needle was inserted at the cranial and medial to the acromion and advanced caudally to the costochondral junction of the first rib. After about 4-5 cm insertion, the stimulator was turned on and was set at the current of 1 mA, 0.2 Hz and 0.1ms. Once the eligible twitches (flexion or extension of the elbow and not pronation of the extremities and twitches of the shoulder) were elicited, the current was gradually decreased to 0.1 mA, 0.2 Hz and 0.1ms (the threshold current) in 0.2 mA increment until the same motor responses with the least current were detected. By negative aspiration for blood or air, the solution was injected slowly and repositioned in a fan-like fashion until all the twitches were disappeared. By accomplishing the procedure, the animals were placed in standing position.

Time to onset and duration of sensory and motor blockade were recorded. Complete sensory blockade was evaluated at antebrachial and interdigital areas (below the elbow) and was considered when responses to superficial and deep pin-prick test using a 25-gauge hypodermic needle as well as pinching of skin using a hemostat (8-inch Rochester Dean Haemostatic Forceps; Martin, Tuttlingen, Germany) closed to the first ratchet were absent. Sensory blockade was assessed every 30 sec until complete sensory blockade was confirmed. Onset of motor blockade was also recorded when the animals were no longer able to bearing their own weights on the affected limb. Duration of sensory and motor blockade was defined as the time elapsed from the complete lack of response until a normal response was exerted. Duration of anesthesia was evaluated every 15 min by the methods stated before.

HR, fR and RT were recorded at base and at 5, 10, 15, 20, 30 min and then every 15 min until 180 min after drugs administration. HR was monitored via thoracic auscultation and fR was counted via chest movement in a 1-min period. RT was measured per rectum using a digital thermometer (AEG, Germany). All injections were applied by the same investigator (H.I.R) and all evaluations were performed by another one (M.K) who was not aware of the treatments.

### Statistical analysis

Statistical analysis was performed by IBM SPSS Statistics for Windows Version 22 (IBM Corporation, NY, USA). The normal distribution of data was confirmed using Kolmogorov-Smirnov test. All data were expressed as mean  $\pm$  standard deviation (SD). Analysis of variance (ANOVA) with repeated measure followed by Bonferroni's test was employed for comparison of the weight, the onset and duration of sensory and motor blockade among treatments as well as HR, fR and RT among and within groups. Comparison of the time to the onset and duration of sensory and motor blockade in each treatment was done using Paired-Sample T Test.  $p < 0.05$  was considered as significant level.

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### Author Contributions

H.I.R: designing and conducting the study, drafting the manuscript; H.N: collecting and analyzing data, reviewing the manuscript; A.A.N: carrying out the experiments, reviewing and revising the manuscript; M.K: carrying out the experiments, collecting and analyzing data

### Conflict of Interest

The authors do not have any potential conflicts of interest to declare.

### References

1. Valverde A, Sinclair M. Ruminant and Swine Local Anesthetic and Analgesic Techniques. In: Grimm KA, Lamont LA, Tranquilli WJ, Greene SA, Robertson SA, editors. *Veterinary Anesthesia and Analgesia*. The Fifth Edition of Lumb and Jones. Pondicherry, India: John Wiley & Sons; 2015;941-959.

esthesia and Analgesia, The Fifth Edition of Lumb and Jones., Pondicherry, India: John Wiley & Sons; 2015;941-959.

2. Skarda R, Tranquilli W. Local and regional anesthetic and analgesic techniques: ruminants and swine. In: Tranquilli WJ, Thurmon JC, Grimm KA, editors. *Lumb and Jones' Veterinary Anesthesia and Analgesia*. 4th ed. Ames, IA: Blackwell Publishing; 2007;643-681.
3. Iwamoto J, Yamagishi N, Sasaki K, Kim D, Devkota B, Furuhashi K. A novel technique of ultrasound-guided brachial plexus block in calves. *Res Vet Sci*. 2012;93:1467-1471.
4. Moens Y. Brachial plexus block in goats using a nerve stimulator. *J Vet Anesth*. 1995;22:39.
5. Estebe J-P, Le Corre P, Chevanne F, Cathelineau G, Le Verge R, Ecoffey C. Motor blockade by brachial plexus block in the sheep. *Anesthesiology*. 2000;93:291-293.
6. Estebe J-P, Le Corre P, Clement R, Du Plessis L, Chevanne F, Le Verge R, et al. Effect of dexamethasone on motor brachial plexus block with bupivacaine and with bupivacaine-loaded microspheres in a sheep model. *Eur J Anesthesiol*. 2003;20:305-310.
7. Estebe J-P, Le Corre P, Du Plessis L, Chevanne F, Cathelineau G, Le Verge R, et al. The pharmacokinetics and pharmacodynamics of bupivacaine-loaded microspheres on a brachial plexus block model in sheep. *Anesth Analg*. 2001;93:447-455.
8. Ghadirian S, Vesal N. Brachial plexus block using lidocaine/epinephrine or lidocaine/xylazine in fat-tailed sheep. *Vet Res Forum*. 2013;4:161-167.
9. Ghadirian S, Vesal N, Maghsoudi B, Akhlagh SH. Comparison of lidocaine, lidocaine-morphine, lidocaine-tramadol or bupivacaine for neural blockade of the brachial plexus in fat-tailed lambs. *Vet Anesth Analg*. 2016;43:109-116.
10. McFarlane I. The lateral approach to pudendal nerve block in the bovine and ovine. *J S Afr Vet Assoc*. 1963;34:73-76.
11. Garcia ER. Local Anesthetics. In: Grimm KA, Lamont LA, Tranquilli WJ, Greene SA, Robertson SA, editors. *Veterinary Anesthesia and Analgesia*, The Fifth Edition of Lumb and Jones. John Wiley & Sons, Inc., Pondicherry, India; 2015;332-354.
12. Sousa AM, Ashmawi HA. Local analgesic effect of tramadol is not mediated by opioid receptors in early postoperative pain in rats. *Braz J Anesthesiol*. (English Edition) 2015;65:186-190.
13. Tsai Y-C, Chang P-J, Jou I-M. Direct tramadol application on sciatic nerve inhibits spinal somatosensory evoked potentials in rats. *Anesth Analg*. 2001;92:1547-1551.
14. Baniadam A, Afshar FS, Ahmadian F. Analgesic effects of tramadol hydrochloride administered via caudal epidural injection in healthy adult cattle. *Am J Vet Res*. 2010;71:720-725.
15. Bigham A, Habibian S, Ghasemian F, Layeghi S. Caudal epidural injection of lidocaine, tramadol, and lidocaine-tramadol for epidural anesthesia in cattle. *J vet pharmacol ther*. 2010;33:439-443.
16. Dehkordi SH, Bigham-Sadegh A, Gerami R. Evaluation of anti-nociceptive effect of epidural tramadol, tramadol-lidocaine and lidocaine in goats. *Vet Anesth Analg*. 2012;39:106-110.
17. Habibian S, Bigham A, Aali E. Comparison of lidocaine, tramadol, and lidocaine-tramadol for epidural analgesia in lambs. *Res Vet Sci*. 2011;91:434-438.
18. Antonucci S. Adjuvants in the axillary brachial plexus blockade. Comparison between clonidine, sufentanil and tramadol. [Abstract] *Minerva Anesthesiol*. 2000;67:23-27.
19. Geze S, Ulusoy H, Ertürk E, Cekic B, Arduc C. Comparison of local anesthetic mixtures with tramadol or fentanyl for axillary plexus block in orthopaedic upper extremity surgery. *Eur J Gen Med*. 2012;9:118-123.
20. Kaabachi O, Ouezini R, Koubaa W, Ghrab B, Zargouni A, Abdelaziz AB. Tramadol as an adjuvant to lidocaine for axillary brachial plexus block. *Anesth Analg*. 2009;108:367-370.
21. Kapral S, Gollmann G, Waltl B, Likar R, Sladen RN, Weinstabl C, et al. Tramadol added to mepivacaine prolongs the duration of an axillary brachial plexus blockade. *Anesth Analg*. 1999;88:853-856.
22. Nagpal V, Rana S, Singh J, Chaudhary SK. Comparative study of systemically and perineurally administered tramadol as an adjunct for supraclavicular brachial plexus block. *J Anesthesiol, Clin pharmacol*. 2015;31:191.
23. Robaux S, Blunt C, Viel E, Cuvillon P, Nouguier P, Dautel G, et al. Tramadol added to 1.5% mepivacaine for axillary brachial plexus block improves post-operative analgesia dose-dependently. *Anesth Analg*. 2004;98:1172-1177.
24. Sarsu S, Mizrak A, Karakurum G. Tramadol use for axillary brachial plexus blockade. *J Surg Res*. 2011;165:e23-e27.
25. Kayser V, Besson J-M, Guilbaud G. Evidence for a noradrenergic component in the antinociceptive effect of the analgesic agent tramadol in an animal model of clinical pain, the arthritic rat. *Eur J Pharmacol*. 1992;224:83-88.
26. Haeseler G, Foadi N, Ahrens J, Dengler R, Hecker H, Leuwer M. Tramadol, fentanyl and sufentanil but not morphine block voltage-operated sodium channels. *Pain*. 2006;126:234-244.
27. Jou I-M, Chu K-S, Chen H-H, Chang P-J, Tsai Y-C. The effects of intrathecal tramadol on spinal somatosensory-evoked potentials and motor-evoked responses in rats. *Anesth Analg*. 2003;96:783-788.
28. Mert T, Gunes Y, Gunay I. Local analgesic efficacy of tramadol following intraplantar injection. *Eur J Pharmacol*. 2007;558:68-72.
29. Wang JT, Chung CC, Whitehead RA, Schwarz SKW, Ries CR, MacLeod BA. Effects of local tramadol administration on peripheral glutamate-induced nociceptive behaviour in mice. *Can J Anesth*. 2010;57:659-663.
30. Alemanno F, Ghisi D, Fanelli A, Fali

dol. [Abstract] *Minerva Anesthesiol*. 2000;67:23-27.

31. Kesimci E, Izdes S, Gozdemir M, Kanbak O. Tramadol does not prolong the effect of ropivacaine 7.5 mg/ml for axillary brachial plexus block. *Acta anesthesiol Scand*. 2007;51:736-741.
32. Sakonju I, Maeda K, Maekawa R, Maebashi R, Kakuta T, Takase K. Relative nerve blocking properties of bupivacaine and ropivacaine in dogs undergoing brachial plexus block using a nerve stimulator. *J Vet Med Sci*. 2009;71:1279-1284.
33. Futema F, Fantoni DT, Auler JOC, Cortopassi SRG, Acaui A, Stopiglia AJ. A new brachial plexus block technique in dogs. *Vet Anesth Analg*. 2002;29:133-139.
34. Skarda R, Tranquilli W. Local anesthetics. In: Tranquilli W, Thurmon J, Grimm K, editors. *Lumb and Jones' Veterinary Anesthesia and Analgesia*. 4th ed. Ames, IA: Blackwell Publishing; 2007;395-418.
35. Budd K. The role of tramadol in acute pain management. *Acute Pain*. 1999;2:189-196.
36. Campoy L, Read MR. The Thoracic Limb. In: Campoy L, Read MR, eds. *Small Animal Regional Anesthesia and Analgesia*. Pondicherry, India: John Wiley & Sons; 2013;141-165.
37. Casati A, Danelli G, Baciarello M, Corradi M, Leone S, Di Cianni S, et al. A prospective, randomized comparison between ultrasound and nerve stimulation guidance for multiple injection axillary brachial plexus block. *Anesthesiology*. 2007;106:992-996.
38. Schoenmakers KP, Wegener JT, Stienstra R. Effect of local anesthetic volume (15 vs 40 mL) on the duration of ultrasound-guided single shot axillary brachial plexus block: a prospective randomized, observer-blinded trial. *Reg Anesth Pain Med*. 2012;37:242-247.
39. Campoy L, Martin-Flores M, Looney AL, Erb HN, Ludders JW, Stewart JE, Gleed RD, Asakawa M. Distribution of a lidocaine-methylene blue solution staining in brachial plexus, lumbar plexus and sciatic nerve blocks in the dog. *Vet Anesth Analg*. 2008;35:348-354.
40. Mahler SP, Adogwa AO. Anatomical and experimental studies of brachial

plexus, sciatic, and femoral nerve-location using peripheral nerve stimulation in the dog. *Vet Anesth Analg*. 2008;35:80-89.